SILYL LINKER FOR SOLID-PHASE SYNTHESIS OF NUCLEIC ACID

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version

FIELD OF THE INVENTION

[0001]

The invention relates to a silyl linker i[illegible]ly introduced on a solid-phase support used for the synthesis of nucleic acid (DNA).

BACKGROUND ART

[0002]

In the progress of diversification of the studies relating to nucleic acids, it is desired to rapidly synthesize with a high purity a functional molecule such as a DNA oligomer liable to oxidative deterioration or a DNA oligomer having a functional moiety unstable under a basic condition, which would be decomposed in such a basic condition as is usually used in DNA synthesis (the treatment with ammonia).

[0003]

Up to now, a benzoic acid-type compound: $iP_2Si-C_6H_4-C(O)-$ type that was developed by one of the present inventors, SEKINE Mitsuo, is known as a silyl linker that can be cut out under a neutral condition (Non-Patent Document 1). However, it was not practically sufficient since it would take such a long time as almost one day to introduce the above compound on a solid-phase support, and an introduction efficiency is as low as 6-8 μmol/g, especially on HCP solid phase having a small

amount of the total amino groups (34 μmol/g).

[0004]

Non-Patent Document 1: Kobori, A.; Miyata, K.; Ushioda, M.; Seio, K.; Sekine, M., Chemistry Letters, 2002, 16-17.

SUMMARY OF THE INVENTION

Problems to be solved by the invention

[0005]

The purpose of the present invention is therefore to develop a silyl linker that can be efficiently introduced on the solid-phase support. The present inventors have studied hard so that the above purpose was accomplished by introducing a spacer into the conventional silyl linker, leading to the present invention.

[0006]

Thus, the present invention relates to a silyl linker for use in the solid-phase synthesis of nucleic acid, comprised of a compound of the general formula or its ester or salt:

$$H-(R1)Si(R2)-(C_6H_4)-CONH-(A)-COOH \quad (I)$$

wherein each of R1 and R2 is an alkyl or aryl group, and (A) represent a spacer moiety.

[0007]

The present invention further relates to a 3'-end nucleoside unit having the above compound linked via an oxygen atom to the 3-position of a sugar of the nucleoside or its derivative wherein, for example, a hydroxy group at 5-position of the sugar

is protected with an appropriate protecting group. The above unit will be especially advantageous when a thymine group is constituting the nucleoside because the thymine has no amino group to be protected in the introduction on the solid-phase.

[0008]

The present invention also relates to a solid-phase support, especially HCP solid-phase support having the above 3'-end nucleoside unit or the above silyl linker for use in the solid-phase synthesis of nucleic acid. The solid-phase support itself is known for those skilled in the art. The present invention also relates to a method for synthesis of a nucleic acid oligomer with the use of the solid-phase support according the present invention. This method is advantageous, especially for the synthesis of a nucleic acid oligomer containing modified bases that are unstable under a basic condition, such as an acetylated cytosine.

Advantages of the invention

[0009]

The silyl linker according to the present invention may be cut out under a neutral condition, and will significantly increase the introduction ratio of the 3'-end nucleoside unit on the solid-phase support up to about 20-30 μmol/g that is thought to be most suitable in the DNA synthesis.

Brief description of drawing

[0010]

Fig. 1 shows a chart in a reverse and anion-exchange chromatography of d[GC^{ac}ATCAGC^{ac}C^{ac}TCAT]synthesized with the use of the silyl linker.

Best Mode for Carrying out the Invention

[0011]

Any moiety known for those skilled in the art may be used as the spacer moiety (A) as long as it can accomplish a desired purpose of the present invention. For example, an alkylene group represented by the formula: $-(CH_2)n-$ (II) wherein "n" is a natural number, preferably 2-18 may be used as the spacer. The alkylene group may have at least one other group such as ether or thioether bond.

[0012]

The silyl group may have any substituents of R1 and R2 known for those skilled in the art, such as, for example, an alkyl group having 1 to 5 carbon atoms or an aryl group such as benzyl, phenyl and naphthyl group, which may have a substituent of the above alkyl, nitro, cyano, halogeno or alkoxy group at any position.

[0013]

Furthermore, the benzene ring structure of the present compound may have any substituent known for those skilled in the art, which, for example, is selected from the group consisting of alkyl having 1 to 4 carbon atoms, halogeno, nitro, cyano and methoxy groups. The groups of "-CONH-" and "Si" are bound to

the benzene ring in a para-position.

[0014]

The ester or salt according to the present invention may be optionally selected from any compounds known for those skilled in the art, which includes triethyl ammonium salt, tributyl ammonium salt and ethyldiisopropyl ammonium salt; and cyanoethylester, allylester and 4-nitrophenylethyl ester.

[0015]

The compound of the present invention may be easily synthesized by those skilled in the art with reference to the following examples. Conditions that are not specifically described in the present specification may be optionally selected by those skilled in the art.

Examples

[0016]

The present invention will be explained more in detail in line with the examples, which should not be construed to impose any limitations on the scope of the present invention.

[0017]

EXAMPLE 1:Synthesis of silyl linker

4-diisopropylsilanylbenzoyl chloride (2)

4-diisopropylsilanyl benzoic acid (1)(6.7g, 28.4 mmol) and thionyl chloride (3.2 mL, 42.6 mmol) were mixed together and heated to reflux for 2 hours. a desired compound was then purified and identified by distillation under a reduced

pressure (1 mmHg, 102-104°C) (5.6 g, 77%). Its NMR data are as follows:

[0018]

^{1}H NMR ($CDCl_3$): 0.95-1.06 (m, 12H), 1.21-1.29 (m, 2H), 3.99 (t, 1H, J = 3.1 Hz), 7.64 (d, 2H, J = 7.8 Hz), 8.03 (d, 2H, J = 7.8 Hz).

^{13}C NMR ($CDCl_3$): 10.7, 18.5, 18.7, 130.0, 133.6, 135.9, 144.5, 168.3.

[0019]

4-[4- (diisopropylsilanyl)benzoylamino]butanoic acid (3)

4-diisopropylsilanylbenzoyl chloride (2)(1.7 g, 6.7 mmol) was added into 1N sodium hydroxide aqueous solution (9 mL) dissolving 4-aminobutanoic acid (910 mg. 8.94 mmol) and stirred for 8 hours. After the addition of 12N hydrochloric acid to the aqueous solution to reach pH 2, the solution was extracted with 400 mL of CH_2Cl_2 and an organic layer was then collected. The resulting organic layer was dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. A desired compound was then purified by silica gel column chromatography. After eluted with chloroform having 0-3 % methanol gradient, the solvent was distilled out to give the desired compound as white solid (1.4 g, 65 %). Its NMR data are as follows:

[0020]

^{1}H NMR ($CDCl_3$): 0.93-1.06 (m, 12H), 1.16-1.25 (m, 2H), 1.94 (t, 2H, J = 4.1 Hz), 2.45 (t, 2H, J = 6.9 Hz), 3.50 (dd, 2H, J =

6.5 Hz, J = 9.7 Hz), 3.93 (t, 1H, J =3.1 Hz), 6.77 (brs, 1H), 7.54 (d, 2H, J = 8.1 Hz), 7.72 (d, 2H, J = 8.1 Hz).

^{13}C NMR ($CDCl_3$): 10.5, 17.9, 18.3, 18.4, 24.3, 31.6, 39.5, 57.9, 77.2, 125.8, 134.3, 135.3, 138.6, 168.3, 176.6.

[0021]

4-[4- (diisopropylsilanyl)benzoylamino]butanoic acid 2-cyanoethyl ester (4)

A condensing agent of N,N-bis (2-oxo-3-oxazolidinyl)-phosphnic acid chloride BOP-Cl (1.5 g, 6.1 mmol) was added to pyridine solution (20 mL)dissolving 4-[4-(diisopropylsilanyl)benzoylamino]butanoic acid (3) (1.3 g, 4.1 mmol), 2-cyanoethanol (548 μL, 8.1 mmol) and triethylamine (828 μL, 6.1 mmol). The resulting mixture was stirred for 3 hours at a room temperature and mixed with water (5 mL). Five minutes later, it was diluted with chloroform (200 mL) and extracted three times with 5 wt% aqueous solution (150 ml)of sodium hydrogen carbonate. An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography. After eluted with chloroform having 0-3 % methanol gradient, the solvent was distilled out to give a desired product (1.2 g, 79 %). Its NMR data are as follows:

[0022]

^{1}H NMR ($CDCl_3$): 0.89-1.00 (m, 12H), 1.15-1.21 (m, 2H), 1.89 (t, 2H, J = 6.9 Hz), 2.40 (t, 2H, J = 7.0 Hz), 2.62 (t, 2H, J = 6.2

Hz), 3.42 (dd, 2H, J = 6.6 Hz, J = 12.8 Hz), 3.89 (t, 1H, J =3.0 Hz), 4.18 (t, 2H, J =6.2 Hz), 6.99 (brs, 1H), 7.49 (d, 2H, J = 7.6 Hz), 7.72 (d, 2H, J = 7.8 Hz).

^{13}C NMR ($CDCl_3$): 10.4, 17.8, 18.3, 18.4, 24.4, 31.2, 39.1, 58.5, 77.2, 116.7, 125.7, 134.7, 135.3, 138.4, 167.4, 172.4.

[0023]

<u>5'-O-(4,4'-dimethoxytrityl)-thymidine-3'-O-diisopropylsilyl-4-benzoylaminobutanoic acid triethylammonium (6)</u>

4-[4- (diisopropylsilanyl)benzoylamino]butanoic acid 2-cyanoethyl ester (4)(1.1 g, 2.9 mmol) was dissolved into anhydrous CH_2Cl_2 (15 mL) and to this solution was added 1,3-dichloro-4,4-dimethylhydantoin (1.2 g, 5.9 mmol). The resulting mixture was stirred for 30 min at a room temperature and mixed into anhydrous CH_2Cl_2 (10 mL) dissolving 5'-O-(4,4'-dimethoxytrityl)-thymidine (3.2 g, 5.9 mmol) and imidazole (2.0 g, 29.4 mmol). The resulting mixture was stirred for 30 min at a room temperature and mixed with water (5 mL). Five minutes later, it was diluted with chloroform (100 mL) and extracted three times with a 5 wt% aqueous solution (150 ml)of sodium hydrogen carbonate. An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography (1% pyridine). After eluted with hexane having 30-100 % chloroform gradient, the solvent was distilled out. The residue was then dissolved in acetonitrile (30 mL),

mixed with DBU (1.7 mL, 11.2 mmol) and stirred for 30 min at a room temperature. The resulting mixture was then mixed with 0.5 M triethyl ammonium carbonate buffer (100 mL) and subjected to extraction with chloroform (100 mL). An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography. After eluted with chloroform comprising 1% triethylamine having 0-3 % methanol gradient, the solvent was distilled out to give a desired product (1.5 g, 54 %). Its NMR data are as follows:

[0024]

^{1}H NMR ($CDCl_3$): 0.92-1.08 (m, 12H), 1.18-1.30 (m, 11H), 1.49 (s, 3H), 1.93 (t, 2H, J = 6.3 Hz), 2.35-2.47 (m, 4H), 3.42 (d, 2H, J = 7.3 Hz), 3.45-3.75 (m, 8H), 3.78 (s, 6H), 4.14 (s, 1H), 4.64 (s, 1H), 6.44 (t, 1H, J = 6.8 Hz), 6.80 (d, 4H, J = 7.6 Hz), 7.18-7.80 (m, 14H).

[0025]

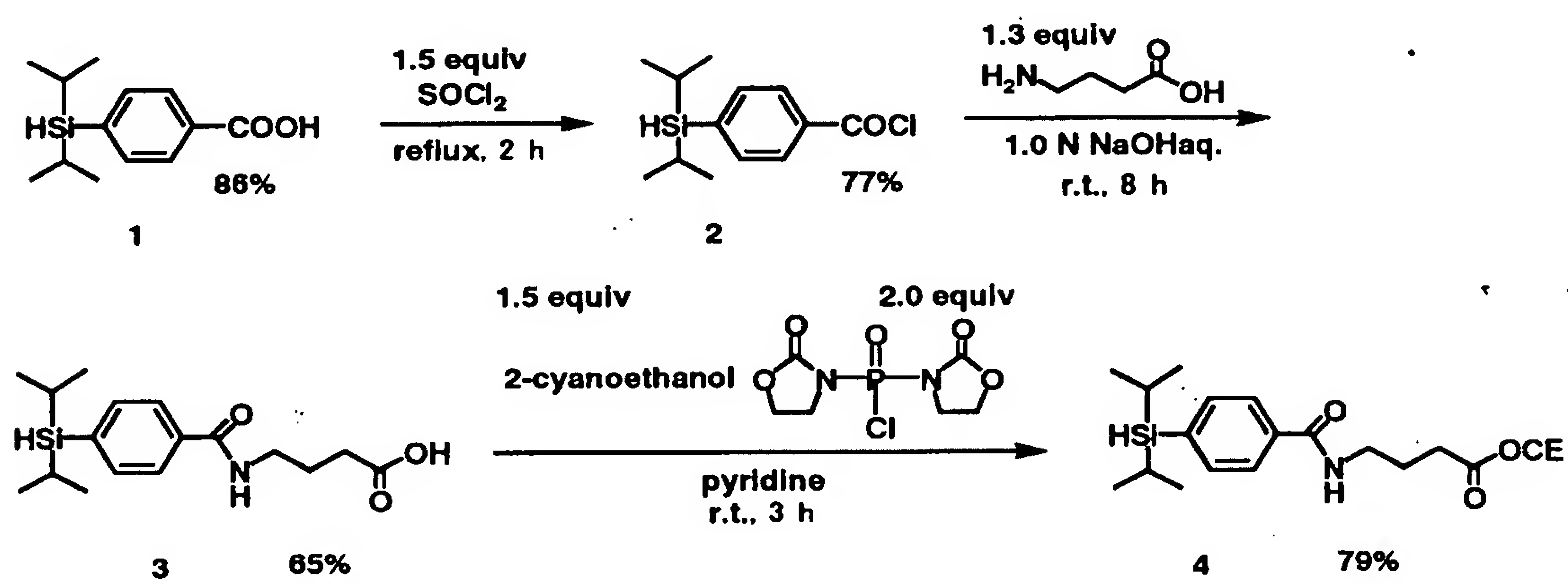
Example 2:Preparation of solid-phase support (7)

Sufficiently dried solid-phase support (highly cross-linked polystyrene:HCP)(500 mg. 52 μmol), 5′-O-(4,4′-dimethoxytrityl)-thymidine-3′-O-diisopropylsilyl-4-benzoylaminobutanoic acid triethylammonium (6)(260 μmol) and DCC (268 mg, 1.3 mmol) were dissolved into dichloromethane (5 mL) and stirred for 12 hours at a room temperature. After the completion of the reaction, the solid-phase support was

filtered, washed with acetonitrile, dried and added to solution made of pyridine (4.5 mL), anhydrous acetic acid (0.5 ml) and DMAP (5 mg). After being stirred for 3 hours, the solid-phase support was filtered again and washed with acetonitrile. The introduction ratio of the compound was measured by colorimetric determination of the trityl group (21 μmol/g). The above synthesis steps were shown in the following chemical formulae 1 and 2.

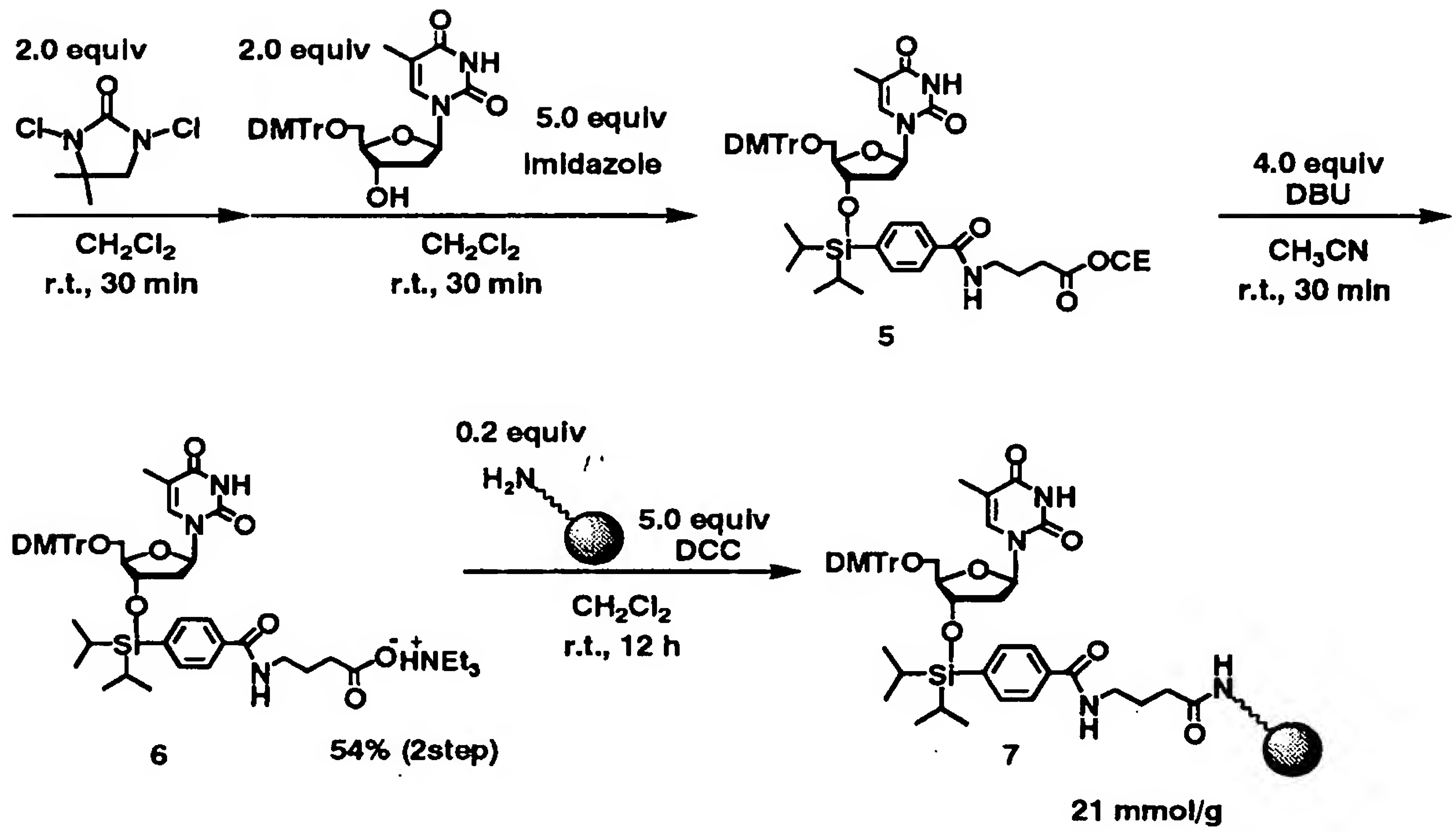
[0026]

[Chemical formula 1]



[0027]

[Chemical formula 2]



[0028]

Example 3:DNA synthesis with the use of the silyl linker

A DNA 13-mer: d[GC^{ac}ATCAGC^{ac}C^{ac}TCAT] wherein the amino groups in some of the cytosine bases were acetylated was synthesized. Such acetyl group was unstable under such a weakly basic condition as ammonia. However, the acetylated cytosine base will form a base pair of Watson-Crick type with a guanine base and a DNA oligomer comprising such acetylated cytosine base will therefore have a specialized property such as a higher forming capacity of a double strand than that comprising a natural cytosine base.

[0029]

The DNA oligomer was automatically synthesized with the use of

the HCP solid-phase support (7) (1 μmol, 21 μmol/g) by means of DNA/RNA Synthesizer 392 (Applied Biosystem Inc.:ABI). Each elongation cycle of the oligomer was shown in TABLE 1 below.

[0030]

[TABLE 1]

Step	operation	Reagent(s)	time, (min)
1	washing	CH_3CN	0.2
2	detritylation	3% Cl_3CCOOH / CH_2Cl_2	1.5
3	washing	CH_3CN	0.4
4	coupling	0.1M amidite + 0.2M $HO^{tf}Bt$ in CH_3CN-NMP (15:1, v/v)	1.0
5	washing	CH_3CN	0.2
6	coupling	0.1M amidite + 0.2M $HO^{tf}Bt$ in CH_3CN-NMP (15:1, v/v)	1.0
7	washing	CH_3CN	0.2
8	oxidation	0.1M I_2 in Py-H_2O-THF (20:2:78, v/v/v)	0.5
9	washing	CH_3CN	0.4

[0031]

The DMTr group was then removed by the treatment with 3 % trichloroacetic acid in CH_2Cl_2 (2 mL) for one minute, and the solid-phase support was washed with CH_2Cl_2 (1 mL x 3) and CH_3CN (1 mL x 3). The cyanoethyl group was then removed by the treatment with 10% DBU in CH_3CN (500 μL). After being washed with CH_3CN (1 mL x 3), the solid-phase support was treated with anhydrous THF solution (500 μL) dissolving TBAF (131 mg, 0.5 mmol) and acetic acid (24 μL, 0.5 mmol) for one hour in order to cut out the DNA oligomer. The resulting mixture solution was desalted with Sep-Pak C18 cartridge, diluted with water and subjected to reverse and anion-exchange HPLC for analysis. The results by mass spectrometry of the resulting compound are as follows: d[GC^{ac}ATCAGC^{ac}C^{ac}TCAT] Mass (M-H) calcd. 4017.72, found

4018.00.

Industrial applicability

[0032]

It will be easy to synthesize DNA derivatives comprising various functional groups that are unstable under the basic condition by using the silyl linker or the 3'-end nucleoside unit according to the present invention.

What is claimed is:

1. A silyl linker for use in the solid-phase synthesis of nucleic acid, comprised of a compound of the general formula or its ester or salt:

$$H-(R1)Si(R2)-(C_6H_4)-CONH-(A)-COOH \quad (I)$$

wherein each of R1 and R2 is an alkyl or aryl group, and (A) represents a spacer moiety.

2. The compound according to Claim 1 wherein the spacer moiety (A) is an alkylene group represented by the formula: $-(CH_2)n-$ wherein "n" is a natural number.

3. The compound according to Claim 2 wherein "n" is 2-18.

4. The compound according to Claim 2 or 3 wherein the alkylene group has at least one ether or thioether bond.

5. The compound according to any one of Claims 1 to 4 wherein R1 and R2 are an alkyl group having 1 to 5 carbon atoms.

6. The compound according to any one of Claims 1 to 4 wherein the aryl group of R1 and R2 has a substituent of alkyl, nitro, cyano, halogeno or methoxy group.

7. The compound according to any one of Claims 1 to 6 wherein a benzene ring structure has a substituent.

8. The compound according to Claim 7 wherein the substituent of the benzene ring structure is selected from the group consisting of alkyl having 1 to 4 carbon atoms, halogeno, nitro, cyano and methoxy groups.

9. A 3'-end nucleoside unit having the compound according to any one of Claims 1 to 8 linked via an oxygen atom to the

3-position of a sugar of the nucleoside or its derivative.

10. The 3'-end nucleoside unit according to Claim 9 wherein a base constituting the nucleoside is thymine.

11. The compound according to Claim 10 which is 5'-O-(4,4'-dimethoxytrityl)—thymidine-3'-O-diisopropylsiyl-4-benzoylaminobutanoic acid triethylammonium.

12. A solid-phase support having the 3'-end nucleoside unit according to Claim 9 introduced thereon.

13. The solid-phase support according to Claim 12 having the 3'-end nucleoside unit at a ratio of 20-30 μmol/g.

14. The solid-phase support according to Claim 12 or 13, which is HCP solid-phase support.

15. A method for synthesis of a nucleic acid oligomer with the use of the solid-phase support according Claims 12, 13 or 14.

16. The method according to Claim 15 wherein the nucleic acid oligomer contains a modified base.